ANADOLU ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ –B
Teorik Bilimler

ANADOLU UNIVERSITY JOURNAL OF SCIENCE AND TECHNOLOGY –B
Theoretical Sciences
Cilt/Vol.: 2-Sayı/No: 1 : 45-62 (2012)

ARAŞTIRMA MAKALESİ / **RESEARCH ARTICLE**

THEORETICAL INVESTIGATION OF CERTAIN TEA COMPOUNDS IN AQUEOUS AND BLOOD PHASES

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ABSTRACT

In this study, certain theoretical properties of tea compounds, such as acidity constant, stability, reactivity, geometry and energy have been calculated prior to laboratory studies to understand the basic principles concerning their behaviors within the human body by MOPAC2009 with the PM6 method. The order of proton dissociation, dipole moment and nucleophilicity for the studied molecules in aqueous and blood phases were investigated. The order of stabilities of the molecules was determined in relation to the Gibbs free energy. It is observed that any molecule having more of the -OH group has more stability and is less reactive. The stabilities of all the molecules are higher in the aqueous phase than those in the blood phase. Their calculated acidity constants were compared with those of experimental values. Positive meaningful correlations ($R^2 > 0.9999$) were observed between thermokinetic pK_a values for **1c, 2ec, 3cg**, **4ecg**, **5egc, 6egcg** and **7ga** in both aqueous and blood phases.

Keywords: Catechins, Polyphenol, Tea, Theoretical calculation, PM6, Marvinbeans, Sparc.

BAZI ÇAY BİLEŞENLERİNİN SU VE KAN FAZINDA TEORİK OLARAK İNCELENMESİ

ÖZ

Bu çalışmada, çay bileşiklerinin asitlik sabiti, kararlılık, reaktivite, geometri ve enerji gibi bazı teorik özellikleri, laboratuvar çalışmalarından önce insan vücudundaki davranışlarının temel prensiplerini anlamak için, MOPAC 2009 (PM6 metodu) ile hesaplanmıştır. Çalışılan bileşiklerin proton verme sıraları, dipol momentleri ve nükleofillikleri su ve kan fazında araştırılmıştır. Moleküllerin kararlılık sıralamaları Gibbs serbest enerjileri dikkate alınarak belirlenmiştir. Daha fazla –OH grubu içeren molekülün daha kararlı ve daha az reaktif olduğu gözlenmiştir. Bütün moleküller, su fazında kan fazına göre daha kararlıdır. Hesaplanan asitlik sabitleri deneysel sabitleriyle karşılaştırılmıştır. **1c, 2ec, 3cg**, **4ecg**; **5egc, 6egcg** ve **7ga** moleküllerinin su ve kan fazındaki termodinamik pK_a değerleri arasında anlamlı bir korelasyon ($R^2 > 0.9999$) gözlenmiştir.

Anahtar Kelimeler: Kateşinler, Polifenol, Çay, Teorik hesaplama, PM6, Marvinbeans, Sparc.

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Recieved: 22 March 2011; **Revised:** 1 November 2011; **Accepted:** 13 November 2011

1. INTRODUCTION

There has been a worldwide increase in both the consumption of tea and the studies of tea beverages, which include biologically active compounds such as catechins and phenolic acids, in recent years. These natural compounds exhibiting strong antioxidant activity have been used as cardiovascular and cancer chemo preventive agents (Shahidi et al. 1992; Yang et al. 2000; Zeeb et al. 2000; Kris-Etherton and Keen 2002; Zaveri 2006; Uysal et al. 2009). The major catechins, such as catechin (**1c**), epicatechin (**2ec**), catechin-gallate (**3cg**), epicatechin-gallate (**4ecg**), epigallocatechin (**5egc**), and epigallocatechin-gallate (**6egcg**) are present in the most-widely consumed green tea, and polyphenols, such as gallic acid (**7ga**) and caffeic acid (**8caffa**), possess anti-inflammatory, anti-microbial, and anti-allergenic activity due to their ability to form complexes with proteins or other biopolymers (Anh et al. 1991; Nakahara et al. 1993; Hatano et al. 1999; Wang et al. 2000). They also function as stress protectors in plant cells by scavenging reactive oxidative species by the photosynthetic electron transport system and, due to their UV-absorbing properties, protect plants from UV radiation from the sun (Kondo et al. 1999). The green tea polyphenols have been recognized as being effective preventers against certain forms of cancer (Yang et al. 2000; Jankun et al. 1997). Although the human epidemiology remains inconclusive, they show cancer preventative effects in some animal models. It is thought that these effects have often been attributed to antioxidant properties. These tea compounds are found in blood and tissues following oral ingestion, preventing human plasma oxidation, and acting as inhibitors of low-density lipoprotein (LDL) oxidation (Yang et al. 1998; Yang et al. 2001). The antioxidant efficiency of these tea compounds has been related to their hydrogen radical donating abilities, and with a number of hydroxyl groups in molecules. Thus, the knowledge of dissociation constants of these molecules is important to understanding of their structure-activity relationship in bioclinic and pharmacological research studies (Ji et al. 2006). These compounds have ionizable hydroxyl groups with pK_a values relatively close to each other. Therefore, knowledge of certain physical properties of these compounds in aqueous and blood phases is crucial.

In laboratory conditions, it is not easy to determine experimentally certain physical constants of these molecules having pK_a values which are relatively close to each other. Therefore, the calculation of these constants by theoretical methods would provide useful data for both academics and nonacademic studies prior to experimental studies to predict their effects on the human body.

To the best of our knowledge, such a comprehensive study has not yet been carried out for tea compounds. However, a theoretical study concerning just catechin and epicatechin has been reported (Ana Marı´a et al. 2006).

The objective of this study is to investigate certain theoretical properties of tea compounds (Fig. 1) such as acidity constant, stability, reactivity, geometry and energy with a fast, easy, cheap, freely online accessible method and without special computer equipment. Then, it is also aimed to compute easily in blood phase the studied compounds on which have not been met any study in the literature and to compare their correlations with aqueous phase.

2. RESULTS AND DISCUSSION

The acidity of a given base for the reaction (1), B, can be calculated according to Eq. (2), where ΔG is the standard free energy:

$$B^{-} + H_3O^{+} \rightleftharpoons BH + H_2O \qquad (1)$$

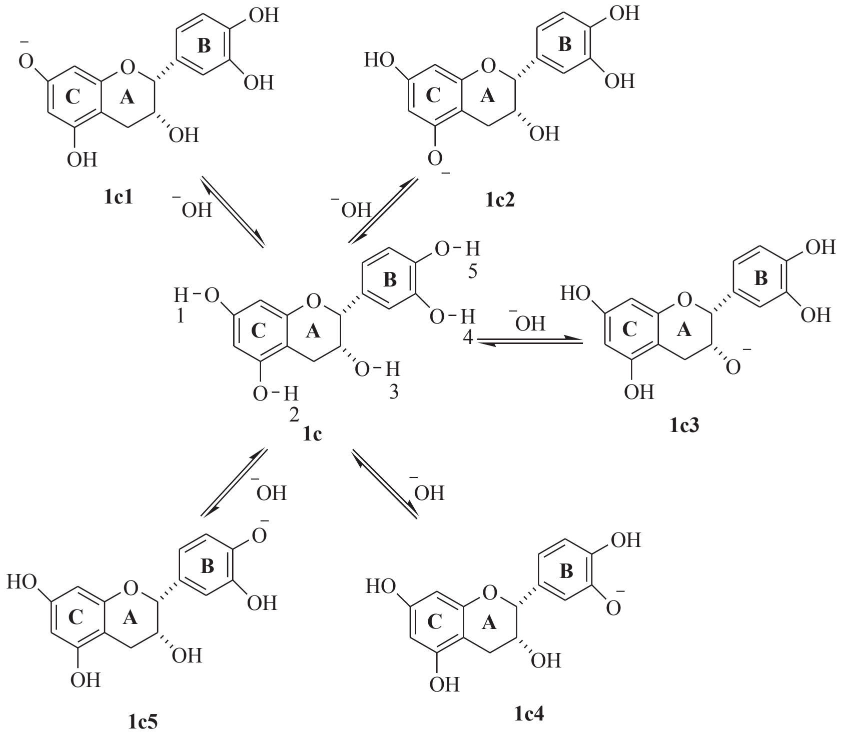
Protonation:

$$\delta\Delta G_{(BH)} = [\Delta G_{(BH)} + \Delta G_{(H2O)}] - [\Delta G_{(B^{-})} + \Delta G_{(H3O+)}] \qquad (2)$$

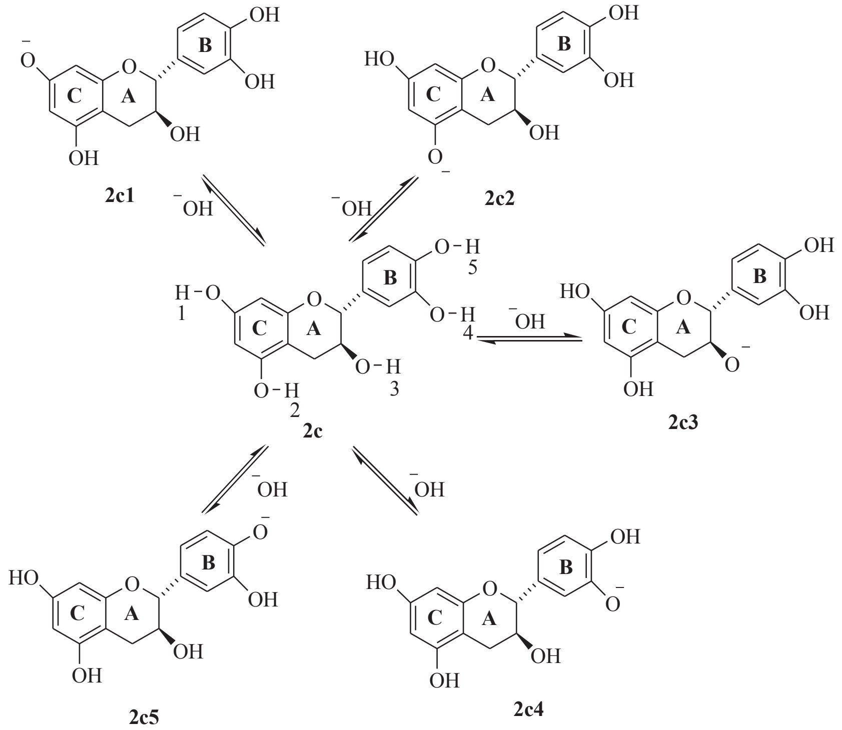
B^- and BH are the anion and protonated species of base B in Eq. (1).
Alternatively, it can be calculated from the thermodynamic free energy relationship:

$$\Delta G = \Delta H - T\Delta S \qquad (3)$$

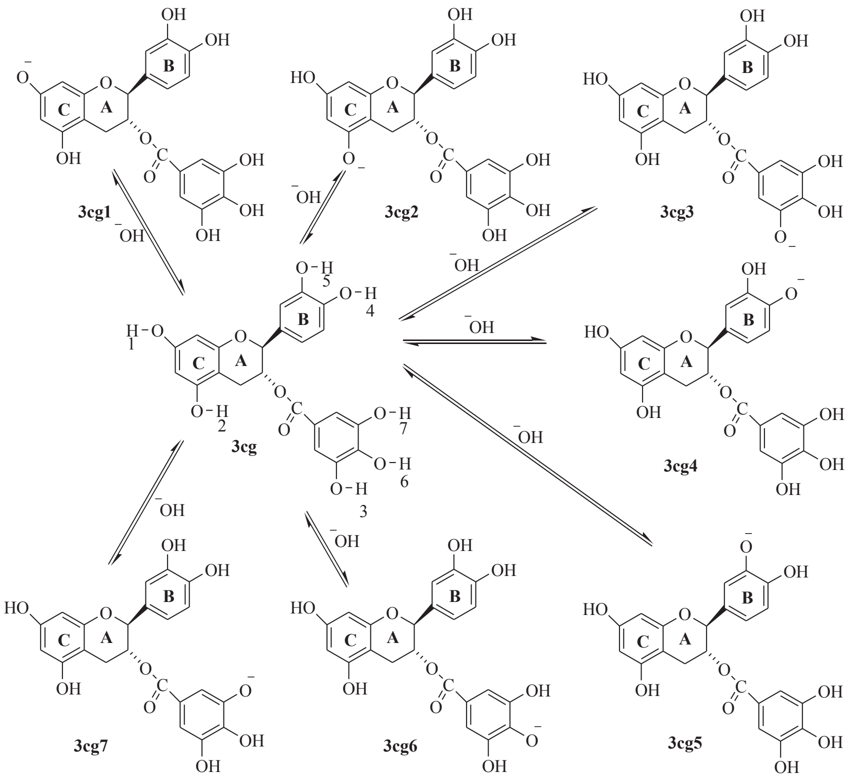
$$\Delta G = -RT\ln K_a \qquad (4)$$



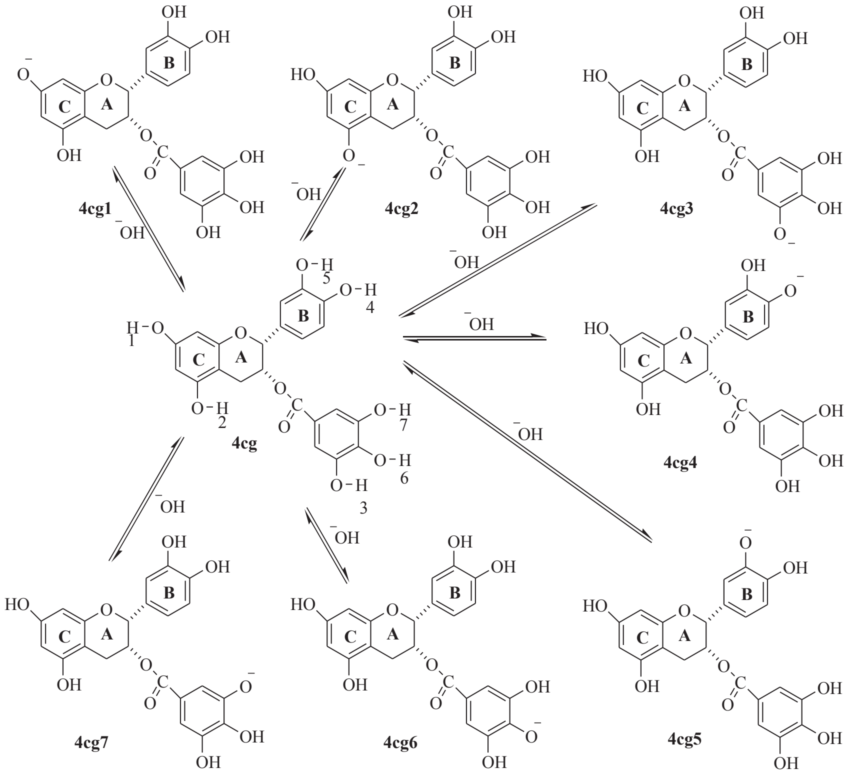
Catechin
(**1c**)



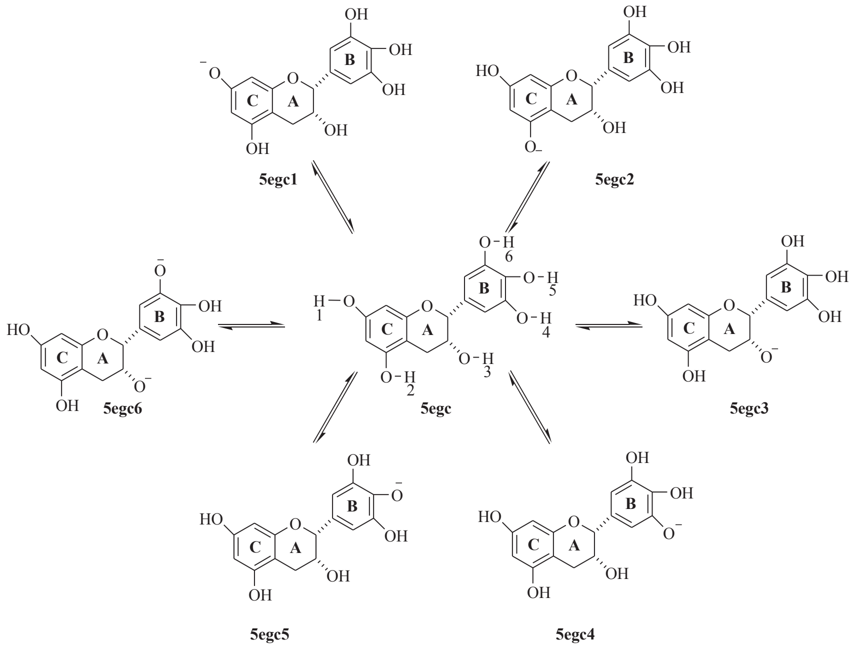
Epicatechin
(**2ec**)



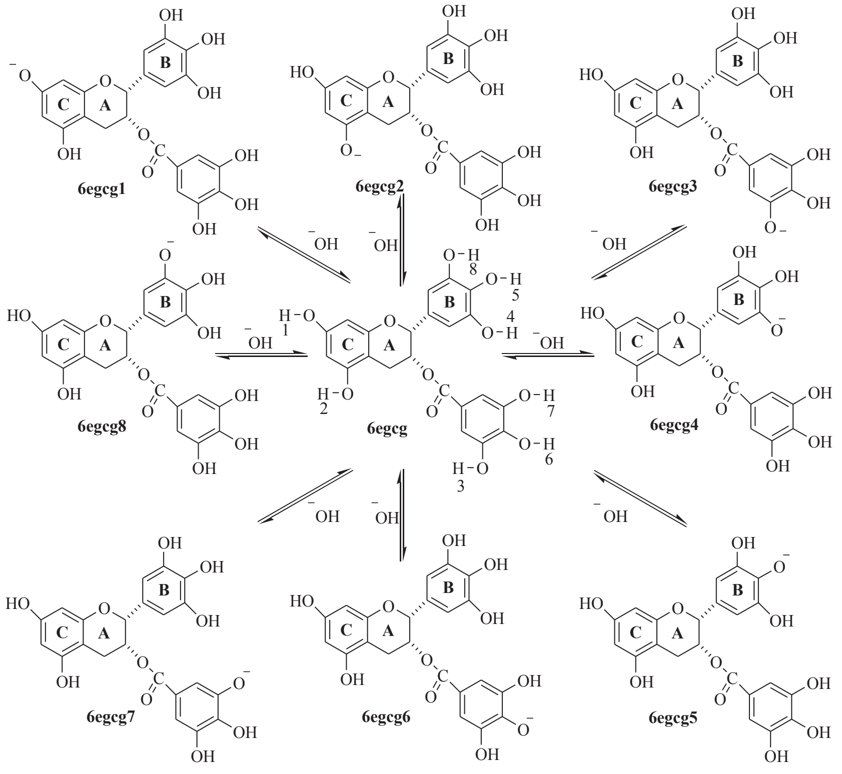
Catechin gallate
(3cg)



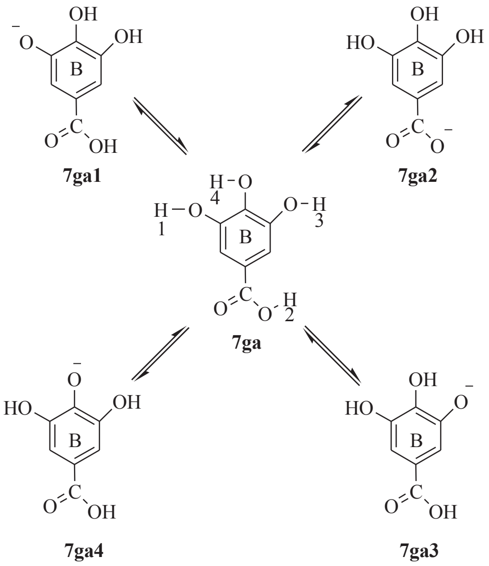
Epicatechin gallate
(4ecg)



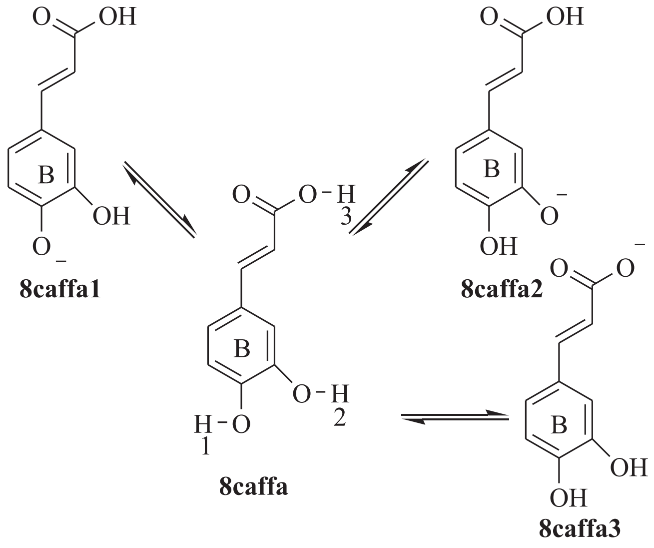
Epigallo catechin
(5egc)



Epigallo catechin gallate
(6egcg)



Gallic acid
(7ga)



Caffeic acid
(8caffa)

Figure 1. The schematic illustrations for the protonation processes of the tea catechins and polyphenols

The self- computed thermodynamic data was used in predicting the pK_a values of various species, using Eq. 5 where $\delta\Delta G_{(BH)}$ is the standard free energy charge for the reaction (1).

$$pK_{a(BH)} = \delta\Delta G_{(BH)} / (2.303RT) \tag{5}$$

(ΔG_f) and (ΔG), standard free energy symbols, represent thermokinetic and thermodynamic data, respectively.

In the theoretical calculations, both aqueous and blood phases were used for determining the activities of catechins and polyphenols in the human metabolism to calculate the most reactive component among these molecules in this study (Table 1).

Table 1. Certain physicochemical parameters of the studied molecules and mono anion forms in aqueous (T=298 K, ε=78.4) and blood (T=310 K, ε=58) phases calculated by the PM6 method

Molecules[a]	**ΔH_f**[b]	**ΔH**[c]	**ΔS**[d]	**ΔG_f**[e]	**ΔG**[f]	**ΔH_f**[b]	**ΔH**[c]	**ΔS**[d]	**ΔG_f**[e]	**ΔG**[f]
	Neutral (BH)-aqueous phase					**Neutral (BH)-blood phase**				
1c	-239.801	13074.095	145.929	-283.288	-30.413	-238.680	14026.146	148.743	-283.005	-30.299
2ec	-243.375	12963.362	143.525	-286.145	-29.807	-242.253	13920.070	146.709	-285.972	-29.799
3cg	-385.150	20049.185	204.660	-446.139	-40.939	-383.467	21486.057	209.482	-445.892	-40.939
4ecg	-384.901	20054.308	202.853	-445.351	-40.396	-383.225	21493.565	207.065	-444.930	-40.212
5egc	-287.474	14022.126	154.391	-333.482	-31.986	-286.279	15030.073	157.769	-333.294	-31.985
6egcg	-429.134	20933.933	213.825	-492.854	-42.786	-427.381	22427.901	219.229	-492.711	-42.902
7ga	-206.948	8314.803	109.713	-239.643	-24.380	-206.271	8878.540	111.563	-239.517	-24.367
8caffa	-151.129	8763.721	113.538	-184.963	-25.071	-150.377	9367.799	115.560	-184.814	-25.069
Mono anion (B$^-$)-aqueous phase						**Mono anion(B$^-$)-blood phase**				
1c1	-335.892	12978.431	146.222	-379.466	-30.596	-334.392	13920.978	149.352	-378.899	-30.586
1c2	-336.078	12990.150	146.017	-379.591	-30.523	-334.604	13922.108	148.973	-378.998	-30.472
1c3	-316.509	12970.805	145.877	-359.980	-30.501	-315.004	13906.298	148.995	-359.404	-30.494
1c4	-332.700	12844.879	143.511	-375.466	-29.921	-331.201	13775.755	146.629	-374.896	-29.920
1c5	-335.663	12989.469	146.859	-379.427	-30.775	-334.226	13927.600	150.158	-378.973	-30.819
2ec1	-335.881	12797.015	143.909	-378.766	-30.088	-334.383	13730.124	147.097	-378.218	-30.105
2ec2	-335.944	12801.506	143.548	-378.721	-29.976	-334.462	13742.350	146.606	-378.151	-29.946
2ec3	-316.557	12943.610	145.952	-360.051	-30.550	-315.055	13880.583	148.941	-359.439	-30.504
2ec4	-332.747	12954.548	146.501	-376.404	-30.703	-331.250	13890.080	148.437	-375.484	-30.344
2ec5	-335.812	12823.458	145.693	-379.228	-30.593	-334.387	13757.568	148.899	-378.759	-30.614
3cg1	-471.355	19479.829	197.371	-530.172	-39.337	-469.421	20893.799	201.481	-529.462	-39.148
3cg2	-476.540	19468.091	198.848	-535.797	-39.788	-474.672	20874.055	202.513	-535.021	-39.475
3cg3	-483.136	18992.383	187.953	-539.146	-37.017	-481.198	20408.536	192.634	-538.603	-36.997
3cg4	-472.481	19509.323	195.468	-530.731	-38.740	-470.575	20918.620	200.242	-530.247	-38.754
3cg5	-478.426	19195.166	192.215	-535.706	-38.085	-476.563	20597.878	196.732	-535.189	-38.028
3cg6	-475.456	19353.347	193.156	-533.016	-38.207	-473.619	20760.485	198.116	-532.658	-38.278
3cg7	-486.034	19257.003	192.847	-543.503	-38.212	-484.188	20667.400	197.685	-543.098	-38.243
4ecg1	-471.687	19498.418	196.943	-530.376	-39.190	-469.730	20914.453	201.584	-529.802	-39.157
4ecg2	-477.680	19393.601	194.409	-535.614	-38.540	-475.789	20806.886	199.188	-535.147	-38.551
4ecg3	-479.132	19651.262	197.093	-537.866	-39.082	-477.218	21062.056	201.719	-537.330	-39.050
4ecg4	-476.620	19402.424	197.228	-535.394	-39.371	-474.713	20804.890	201.806	-534.851	-39.333
4ecg5	-474.961	19360.941	192.829	-532.424	-38.102	-473.112	20766.840	197.526	-531.975	-38.096
4ecg6	-479.268	19455.850	198.146	-538.315	-39.592	-477.386	20862.539	202.368	-537.692	-39.443
4ecg7	-478.183	18949.967	186.040	-533.623	-36.490	-476.252	20361.693	190.747	-533.095	-36.481
5egc1	-380.671	13984.596	152.897	-426.234	-31.579	-379.114	14986.702	156.187	-425.658	-31.557
5egc2	-380.671	13984.596	152.897	-426.234	-31.579	-379.068	15032.116	155.367	-425.367	-31.267
5egc3	-361.644	13460.179	147.480	-405.593	-30.489	-360.090	14456.724	150.877	-405.051	-30.505
5egc4	-382.393	13775.795	151.444	-427.523	-31.354	-380.899	14772.058	154.927	-427.067	-31.396
5egc5	-377.537	13902.378	152.519	-422.988	-31.548	-375.998	14899.220	155.877	-422.449	-31.552
5egc6	-380.031	13778.410	151.625	-425.215	-31.406	-378.471	14771.054	155.035	-424.671	-31.429

Table 1. (Continued) Certain physicochemical parameters of the studied molecules and mono anion forms in aqueous (T=298 K, ε=78.4) and blood (T=310 K, ε=58) phases calculated by the PM6 method

6egcg1	-520.014	20707.281	207.079	-581.723	-41.002	-517.950	22188.203	212.635	-581.315	-41.177
6egcg2	-521.136	20258.753	200.287	-580.822	-39.427	-519.197	21717.718	205.019	-580.293	-39.378
6egcg3	-519.750	20021.323	198.268	-578.834	-39.062	-517.736	21484.926	203.037	-578.241	-39.020
6egcg4	-514.503	20557.285	202.014	-574.703	-39.643	-512.463	22033.251	207.186	-574.204	-39.708
6egcg5	-523.320	20288.472	200.768	-583.149	-39.540	-521.366	21754.615	205.718	-582.670	-39.549
6egcg6	-524.640	20322.005	201.958	-584.824	-39.862	-522.641	21792.862	207.019	-584.333	-39.899
6egcg7	-525.130	20379.775	204.348	-586.026	-40.516	-523.172	21842.428	208.134	-585.196	-40.181
6egcg8	-531.073	20353.635	202.515	-591.422	-39.996	-529.159	21832.769	208.937	-591.422	-40.430
7ga1	-304.427	7873.122	105.755	-335.942	-23.642	-303.408	8405.959	107.505	-335.444	-23.630
7ga2	-306.558	7745.196	104.684	-337.754	-23.451	-305.472	8282.691	106.512	-337.213	-23.458
7ga3	-303.076	7871.621	107.744	-335.184	-24.236	-302.016	8407.490	109.444	-334.630	-24.207
7ga4	-304.004	7834.405	106.641	-335.783	-23.944	-302.957	8367.778	108.325	-335.238	-23.913
8caffa1	-246.797	8286.870	111.706	-280.085	-25.001	-245.783	8855.535	113.573	-279.628	-24.989
8caffa2	-241.906	8134.321	109.337	-274.488	-24.448	-240.851	8700.298	111.211	-273.992	-24.441
8caffa3	-250.267	8394.615	111.825	-283.591	-24.929	-249.163	8969.410	113.708	-283.048	-24.915
H_2O	-61.357	2373.258	45.108	-74.799	-11.069	-61.136	2376.435	45.005	-74.547	-11.035
H_3O^+	48.753	2383.074	46.419	34.920	-11.450	58.582	2427.178	44.734	45.251	-10.904

[a]:The last number of the symbols of the molecules represents leaving –H atom numbers marked in Fig. 1

[b] ΔH_f: Heat of formation;

[c] ΔH: Enthalpy; and

[d] ΔS: Entropy were calculated by MOPAC2009.

[e] ΔG_f: Formation Gibbs free energy was calculated by the equation of $\Delta G_f = \Delta H_f - T\Delta S$

[f] ΔG: Gibbs free energy was calculated by $\Delta G = \Delta H - T\Delta S$

2.1 Gibbs Free Energy (ΔG and ΔG_f)

As can be seen from Table 1, the order of stabilities of these molecules depends on their Gibbs Free Energies (ΔG and ΔG_f) in the blood phase being the same as those in the aqueous phase. It is observed that any molecule having more of the -OH group has more stability and is less reactive. It is thought that the reason for this originates from a possible solvation. When the stabilites in the aqueous and blood phases are examined it is realized that they are close to each other in two phases but the stabilities of all the molecules are higher in the aqueous phase than those in the blood phase. This case results from the higher solvation strength of the water. The best reactive structures are 8caffa and 7ga among these molecules. In addition to these findings, the ΔG order for 8caffa and the 7ga are different from those for the ΔG_f order. It is thought that 8caffa in the order of ΔG is dominant kinetically and 7ga in the order of ΔG_f is dominant thermodynamically.

Certain catechin molecules have epimers. In fact, this situation which has its origins in molecular geometry is crucial for human metabolism. In the aqueous and blood phases, **2ec** (including 5 -OH group) and **3cg** (including 7 -OH group) are more stable than **1c** (having 5 -OH) and **4ecg** (having 7 -OH group), respectively.

In Fig. 1 an ‘**A**’ ring represents a saturated six carbon ring for the studied molecules and this ring plays an important role from the point of view of stability in the configuration. The ‘**A**’ ring does not lie in one plane. Thus, the ring prefers the most stable conformation. **2ec** and **4ecg**, epimer couples, placed on the more stable position than **1c** and **3cg**. **1c** and **3cg** placed in the steric affected position. Even if the energies of these four molecules are not too different from each other, **3cg** and **4ecg** are more stable than **1c** and **2ec** and their reactivities are less than **1c** and **2ec**.

1c (having 5 –OH group), **2ec** (having 5-OH group), **5egc** (having 6 –OH group), **3cg** (having 7 -OH group), **4ecg** (having 7 –OH group) and **6egcg** (having 8 –OH group) have the same structure. But, the '**B**' ring in **5egc** and **6egcg** has one more -OH than the others. The stability order is **6egcg**>**3cg**>**4ecg**>**5egc**>**2ec**>**1c** in both the aqueous and the blood phases. **6egcg**, **3cg** and **4ecg** are more stable, less reactive and have less biologically activity than **5egc**, **2ec** and **1c**.

7ga and **8caffa** are the most reactive molecules among the studied compounds. The structures of these molecules are totally different from the other molecules. **7ga** and **8caffa** include the least –OH group among the studied catechins. Since they have one ring, their reactivities are higher than the catechins.

In conclusion, it is thought that not only the number of –OH group but also the geometry of the tea components has an important role in the reactivities of these compounds in human metabolism.

Table 2. Certain physical parameters of the studied molecules in the aqueous (T=298 K, ε=78.4) and blood (T=310 K, ε=58) phases

Molecules	**HOMO**	**LUMO**	**N**[a]	**D**[b]	**HOMO**	**LUMO**	**N**[a]	**D**[b]
	Aqueous phase				**Blood phase**			
1c	-9.066	-0.322	-8.744	5.764	-9.064	-0.320	-8.744	5.747
2ec	-9.125	-0.434	-8.691	4.587	-9.125	-0.434	-8.691	4.573
3cg	-9.175	-1.287	-7.888	7.193	-9.175	-1.286	-7.889	7.172
4ecg	-9.163	-1.312	-7.851	6.705	-9.161	-1.312	-7.849	6.689
5egc	-9.449	-0.595	-8.854	5.127	-9.448	-0.595	-8.853	5.115
6egcg	-9.457	-1.290	-8.167	4.963	-9.454	-1.288	-8.166	4.951
7ga	-9.444	-1.272	-8.172	5.707	-9.443	-1.271	-8.172	5.690
8caffa	-9.090	-1.265	-7.825	6.664	-9.091	-1.265	-7.826	6.652

[a] N=HOMO-LUMO
[b] D: Dipole moment

2.2 Dipole Moment

When the dipole moment (**D**) of the molecules was investigated, no important correlation could be observed between the molecule structures and reactivities for all of the studied molecules (Table 2). It is thought that the dipole moment does not give any important information from the viewpoint of the reactivity for these kinds of molecules in the present study.

2.3 Nucleophilicity

When the nucleophilicities **(N** =HOMO-LUMO**)** of the molecules are investigated, the **N** value order is given in the following order: **5egc** (including the 6-OH group)>**1c** (including 5 –OH group) >**2ec** (including 5 –OH group) and **6egcg** (including 8 -OH group)>**3cg** (including 7 -OH group) >**4ecg** (including 7 –OH group). But, the **N** values of **7ga** and **8caffa** are close to catechins. It is therefore thought that the nucleophilicity does not show an important effect on determining the reactivity.

2.4 $\mathrm{pK_a}$ (PM6, Thermodynamic, Thermokinetic, Marvinbeans, Sparc)

The $\mathrm{pK_a}$ values of the tea compounds were computed by PM6 (automatically computed by MOPAC2009), Thermodynamic (ΔG), Thermokinetic (ΔG_f), Marvinbeans and Sparc methods (Table 3-5).

As it can be seen from Fig. 2 and Fig. 3, the $\mathrm{pK_a}$ values, calculated using different methods [PM6, ΔG_f (Thermokinetic), ΔG (Thermodynamic), Marvinbeans and Sparc], were compared with those of the experimental ones in both the aqueous and the blood phases. The pK_a values were compared by computation with different methods in both aqueous and blood phases; the best correlations could be observed between PM6 and experimental pK_a values for **6egcg and 8caffa**; PM6-ΔG_f for **4ecg**; Marvin-experimental for **1c and 2ec;** ΔG_f-Sparc for **3cg**, ΔG_f-ΔG for **5egc** and Marvinbeans-Sparc for **7ga** in the aqueous phase; and Marvinbeans-experimental for **1c** and **2ec**; ΔG_f-Marvinbeans for **3cg**; ΔG-experimental for **4ecg**; ΔG_f-Sparc for **5egc**; ΔG-Sparc for **6egcg**; and Marvinbeans-Sparc for **7ga** and ΔG_f-experimental for **8caffa** in the blood phase.

A good correlation could not be observed among the pK_a values for the similar acidic proton belonging to the –OH in the molecules for all calculation methods [PM6, ΔG_f (Thermokinetic), ΔG (Thermodynamic), Marvinbeans and Sparc] (Table 5).

Positive meaningful correlations ($R^2 > 0.9999$) were observed between thermokinetic pK_a values both in blood and aqueous phases for **1c, 2ec, 3cg**, **4ecg**; **5egc, 6egcg** and **7ga** (any table or figure was not given here).

Table 3. Thermokinetic pK_a values of the molecules in the aqueous (T=298 K, ε=78.4) and blood (T=310 K, ε=58) phases

Protonation process[a]	**$\Delta G_f(B^-)$**[b]	**$\Delta G_f(BH)$**[c]	**$\delta\Delta G_f(BH)$**[d]	**$pK_{af}(BH)$**[e]	**$\Delta G_f(B^-)$**[b]	**$\Delta G_f(BH)$**[c]	**$\delta\Delta G_f(BH)$**[d]	**$pK_{af}(BH)$**[e]
	Aqueous phase				**Blood phase**			
1c-1c1	-379.466	-283.288	13.541	9.930	-378.899	-283.005	23.904	17.530
1c-1c2	-379.591	-283.288	13.416	9.838	-378.998	-283.005	23.805	17.457
1c-1c3	-359.980	-283.288	33.027	24.219	-359.404	-283.005	43.399	31.825
1c-1c4	-375.466	-283.288	17.541	12.863	-374.896	-283.005	27.907	20.465
1c-1c5	-379.427	-283.288	13.580	9.958	-378.973	-283.005	23.830	17.475
2ec-2ec1	-378.766	-286.145	17.098	12.539	-378.218	-285.972	27.552	20.205
2ec-2ec2	-378.721	-286.145	17.143	12.571	-378.151	-285.972	27.620	20.254
2ec-2ec3	-360.051	-286.145	35.814	26.263	-359.439	-285.972	46.331	33.975
2ec-2ec4	-376.404	-286.145	19.460	14.271	-375.484	-285.972	30.286	22.209
2ec-2ec5	-379.228	-286.145	16.636	12.199	-378.759	-285.972	27.011	19.808
3cg-3cg1	-530.172	-446.139	25.686	18.836	-529.462	-445.892	36.228	26.567
3cg-3cg2	-535.797	-446.139	20.061	14.711	-535.021	-445.892	30.670	22.491
3cg-3cg3	-539.146	-446.139	16.712	12.255	-538.603	-445.892	27.087	19.864
3cg-3cg4	-530.731	-446.139	25.127	18.426	-530.247	-445.892	35.443	25.991
3cg-3cg5	-535.706	-446.139	20.151	14.777	-535.189	-445.892	30.501	22.367
3cg-3cg6	-533.016	-446.139	22.841	16.750	-532.658	-445.892	33.033	24.224
3cg-3cg7	-543.503	-446.139	12.355	9.060	-543.098	-445.892	22.592	16.567
4ecg-4ecg1	-530.376	-445.351	24.694	18.109	-529.802	-444.930	34.926	25.612
4ecg-4ecg2	-535.614	-445.351	19.456	14.268	-535.147	-444.930	29.581	21.693
4ecg-4ecg3	-537.866	-445.351	17.205	12.616	-537.330	-444.930	27.398	20.091
4ecg-4ecg4	-535.394	-445.351	19.676	14.429	-534.851	-444.930	29.877	21.909
4ecg-4ecg5	-532.424	-445.351	22.646	16.607	-531.975	-444.930	32.753	24.019
4ecg-4ecg6	-538.315	-445.351	16.755	12.287	-537.692	-444.930	27.037	19.826
4ecg-4ecg7	-533.623	-445.351	21.447	15.728	-533.095	-444.930	31.634	23.198
5egc-5egc1	-426.234	-333.482	16.967	12.442	-425.658	-333.294	27.435	20.118
5egc-5egc2	-426.234	-333.482	16.967	12.442	-425.367	-333.294	27.725	20.331
5egc-5egc3	-405.593	-333.482	37.609	27.579	-405.051	-333.294	48.041	35.229

Table 3. (Continued) Thermokinetic pK_a values of the molecules in the aqueous (T=298 K, ε=78.4) and blood (T=310 K, ε=58) phases

5egc-5egc4	-427.523	-333.482	15.678	11.497	-427.067	-333.294	26.025	19.085
5egc-5egc5	-422.988	-333.482	20.214	14.823	-422.449	-333.294	30.643	22.471
5egc-5egc6	-425.215	-333.482	17.986	13.190	-424.671	-333.294	28.421	20.842
6egcg-6egcg1	-581.723	-492.854	20.849	15.289	-581.315	-492.711	31.194	22.875
6egcg-6egcg2	-580.822	-492.854	21.751	15.951	-580.293	-492.711	32.217	23.625
6egcg-6egcg3	-578.834	-492.854	23.739	17.408	-578.241	-492.711	34.268	25.129
6egcg-6egcg4	-574.703	-492.854	27.870	20.437	-574.204	-492.711	38.305	28.089
6egcg-6egcg5	-583.149	-492.854	19.424	14.244	-582.670	-492.711	29.839	21.881
6egcg-6egcg6	-584.824	-492.854	17.749	13.016	-584.333	-492.711	28.176	20.662
6egcg-6egcg7	-586.026	-492.854	16.547	12.134	-585.196	-492.711	27.313	20.029
6egcg-6egcg8	-591.422	-492.854	11.150	8.177	-591.422	-492.711	21.087	15.463
7ga-7ga1	-335.942	-239.643	13.420	9.841	-335.444	-239.517	23.870	17.505
7ga-7ga2	-337.754	-239.643	11.608	8.512	-337.213	-239.517	22.102	16.208
7ga-7ga3	-335.184	-239.643	14.178	10.397	-334.630	-239.517	24.685	18.102
7ga-7ga4	-335.783	-239.643	13.579	9.957	-335.238	-239.517	24.077	17.656
8caffa-8caffa1	-280.085	-184.963	14.597	10.704	-279.628	-184.814	24.984	18.321
8caffa-8caffa2	-274.488	-184.963	20.194	14.809	-273.992	-184.814	30.620	22.454
8caffa-8caffa2	-283.591	-184.963	11.092	8.134	-283.048	-184.814	21.564	15.813

[a]: These symbols represent the protonation process for the molecules given in Fig.I
[b]**$\Delta G_f(B^-)$**: Formation Gibbs free energy of **B**$^-$
[c] **$\Delta G_f(BH)$:** Formation Gibbs free energy of protonated **BH**
[d] **$\delta\Delta G_f(BH)$:** Gibbs free energy calculated according to the Eq. 2 for the reaction (1)
[e] **pK_{af}(BH):** Thermokinetic pK_a values calculated according to the Eq. 5

Table 4. Thermodynamic pK_a values of the molecules in the aqueous (T=298 K, ε=78.4) and blood (T=310 K, ε=58) phases

Protonation process[a]	**$\Delta G(B^-)$**[b]	**$\Delta G(BH)$**[c]	**$\delta\Delta G(BH)$**[d]	**pK_a(BH)**[e]	**$\Delta G(B^-)$**[b]	**$\Delta G(BH)$**[c]	**$\delta\Delta G(BH)$**[d]	**pK_{af}(BH)**[e]
	Aqueous phase				**Blood phase**			
1c-1c1	-30.596	-30.413	-0.564	-0.413	-30.586	-30.299	-0.418	-0.306
1c-1c2	-30.523	-30.413	-0.491	-0.360	-30.472	-30.299	-0.304	-0.223
1c-1c3	-30.501	-30.413	-0.469	-0.344	-30.494	-30.299	-0.326	-0.239
1c-1c4	-29.921	-30.413	0.111	0.081	-29.920	-30.299	0.248	0.182
1c-1c5	-30.775	-30.413	-0.743	-0.545	-30.819	-30.299	-0.651	-0.478
2ec-2ec1	-30.088	-29.807	-0.662	-0.485	-30.105	-29.799	-0.437	-0.320
2ec-2ec2	-29.976	-29.807	-0.550	-0.403	-29.946	-29.799	-0.278	-0.204
2ec-2ec3	-30.550	-29.807	-1.124	-0.824	-30.504	-29.799	-0.836	-0.613
2ec-2ec4	-30.703	-29.807	-1.277	-0.936	-30.344	-29.799	-0.676	-0.496
2ec-2ec5	-30.593	-29.807	-1.167	-0.856	-30.614	-29.799	-0.946	-0.694
3cg-3cg1	-39.337	-40.939	1.222	0.896	-39.148	-40.939	1.661	1.218
3cg-3cg2	-39.788	-40.939	0.770	0.565	-39.475	-40.939	1.334	0.978
3cg-3cg3	-37.017	-40.939	3.541	2.597	-36.997	-40.939	3.812	2.795
3cg-3cg4	-38.740	-40.939	1.818	1.333	-38.754	-40.939	2.055	1.507

Table 4. (Continued) Thermodynamic pK$_a$ values of the molecules in the aqueous (T=298 K, ε=78.4) and blood (T=310 K, ε=58) phases

3cg-3cg5	-38.085	-40.939	2.473	1.814	-38.028	-40.939	2.780	2.039
3cg-3cg6	-38.207	-40.939	2.351	1.724	-38.278	-40.939	2.530	1.856
3cg-3cg7	-38.212	-40.939	2.347	1.721	-38.243	-40.939	2.566	1.881
4ecg-4ecg1	-39.190	-40.396	0.824	0.605	-39.157	-40.212	0.923	0.677
4ecg-4ecg2	-38.540	-40.396	1.475	1.081	-38.551	-40.212	1.530	1.122
4ecg-4ecg3	-39.082	-40.396	0.932	0.684	-39.050	-40.212	1.030	0.756
4ecg-4ecg4	-39.371	-40.396	0.643	0.472	-39.333	-40.212	0.748	0.548
4ecg-4ecg5	-38.102	-40.396	1.913	1.403	-38.096	-40.212	1.985	1.455
4ecg-4ecg6	-39.592	-40.396	0.423	0.310	-39.443	-40.212	0.638	0.468
4ecg-4ecg7	-36.490	-40.396	3.525	2.585	-36.481	-40.212	3.600	2.640
5egc-5egc1	-31.579	-31.986	0.027	0.020	-31.557	-31.985	0.297	0.218
5egc-5egc2	-31.579	-31.986	0.027	0.020	-31.267	-31.985	0.587	0.431
5egc-5egc3	-30.489	-31.986	1.117	0.819	-30.505	-31.985	1.349	0.990
5egc-5egc4	-31.354	-31.986	0.251	0.184	-31.396	-31.985	0.458	0.336
5egc-5egc5	-31.548	-31.986	0.057	0.042	-31.552	-31.985	0.302	0.221
5egc-5egc6	-31.406	-31.986	0.199	0.146	-31.429	-31.985	0.425	0.312
6egcg-6egcg1	-41.002	-42.786	1.403	1.029	-41.177	-42.902	1.594	1.169
6egcg-6egcg2	-39.427	-42.786	2.978	2.184	-39.378	-42.902	3.393	2.488
6egcg-6egcg3	-39.062	-42.786	3.342	2.451	-39.020	-42.902	3.751	2.751
6egcg-6egcg4	-39.643	-42.786	2.762	2.025	-39.708	-42.902	3.063	2.246
6egcg-6egcg5	-39.540	-42.786	2.864	2.100	-39.549	-42.902	3.222	2.363
6egcg-6egcg6	-39.862	-42.786	2.543	1.865	-39.899	-42.902	2.872	2.106
6egcg-6egcg7	-40.516	-42.786	1.889	1.385	-40.181	-42.902	2.590	1.899
6egcg-6egcg8	-39.996	-42.786	2.409	1.767	-40.430	-42.902	2.341	1.717
7ga-7ga1	-23.642	-24.380	0.357	0.262	-23.630	-24.367	0.606	0.444
7ga-7ga2	-23.451	-24.380	0.548	0.402	-23.458	-24.367	0.778	0.571
7ga-7ga3	-24.236	-24.380	-0.237	-0.174	-24.207	-24.367	0.030	0.022
7ga-7ga4	-23.944	-24.380	0.054	0.040	-23.913	-24.367	0.323	0.237
8caffa-8caffa1	-25.001	-25.071	-0.312	-0.229	-24.989	-25.069	-0.051	-0.037
8caffa-8caff2	-24.448	-25.071	0.242	0.177	-24.441	-25.069	0.498	0.365
8caffa-8caffa2	-24.929	-25.071	-0.240	-0.176	-24.915	-25.069	0.023	0.017

[a]: These symbols represent the protonation process for the molecules given in Fig.1

[b] **ΔG(B$^-$)**: Formation Gibbs free energy of **B$^-$**

[c] **ΔG(BH):** Formation Gibbs free energy of protonated **BH**

[d] **$\delta\Delta$G(BH):** Gibbs free energy calculated according to the Eq. 2 for the reaction (1)

[e] **pK$_a$(BH):** Thermokinetic pK$_a$ values calculated according to the Eq. 5

Table 5. pK_a order belonging to the -OH group protons in the structure of molecules calculated from the different methods

PM6 (directly calculated by MOPAC2009 using pK_a keyword)															
1c		**2ec**		**3cg**		**4ecg**		**5egc**		**6egcg**		**7ga**		**8caffa**	
1-H	8.644	**1-H**	8.644	**6-H**	7.881	**6-H**	7.881	**2-H**	8.415	**6-H**	7.832	**2-H**	3.942	**3-H**	3.644
2-H	9.333	**2-H**	9.333	**2-H**	8.374	**2-H**	8.374	**6-H**	9.020	**2-H**	8.237	**1-H**	8.865	**2-H**	9.276
5-H	10.748	**5-H**	10.748	**5-H**	9.170	**5-H**	9.170	**1-H**	10.584	**4-H**	8.857	**3-H**	11.228	**1-H**	12.690
4-H	12.647	**4-H**	12.647	**1-H**	10.248	**1-H**	10.248	**4-H**	11.245	**1-H**	10.011	**4-H**	14.817		
3-H	14.089	**3-H**	14.089	**7-H**	11.417	**7-H**	11.417	**3-H**	13.942	**8-H**	10.590				
				4-H	12.340	**4-H**	12.340	**5-H**	14.746	**3-H**	11.459				
				3-H	13.138	**3-H**	13.138			**7-H**	13.011				
										5-H	13.735				
ΔG$_f$ (Thermokinetic)															
1c		**2ec**		**3cg**		**4ecg**		**5egc**		**6egcg**		**7ga**		**8caffa**	
2-H	9.838	**5-H**	12.199	**7-H**	9.060	**6-H**	12.287	**4-H**	11.497	**8-H**	8.177	**2-H**	8.512	**3-H**	8.134
1-H	9.930	**4-H**	14.271	**3-H**	12.255	**3-H**	12.616	**1-H**	12.442	**7-H**	12.134	**1-H**	9.841	**1-H**	10.704
5-H	9.958	**1-H**	12.539	**2-H**	14.711	**2-H**	14.268	**2-H**	12.442	**6-H**	13.016	**4-H**	9.957	**2-H**	14.809
4-H	12.863	**2-H**	12.571	**5-H**	14.777	**4-H**	14.429	**6-H**	13.190	**5-H**	14.244	**3-H**	10.397		
3-H	24.219	**3-H**	26.263	**6-H**	16.750	**7-H**	15.728	**5-H**	14.823	**1-H**	15.289				
				4-H	18.426	**5-H**	16.607	**3-H**	27.579	**2-H**	15.951				
				1-H	18.836	**1-H**	18.109			**3-H**	17.408				
										4-H	20.437				
ΔG (Thermodynamic)															
	1c		**2ec**		**3cg**		**4ecg**		**5egc**		**6egcg**		**7ga**		**8caffa**
5-H	-0.545	**4-H**	-0.936	**2-H**	0.565	**6-H**	0.310	**1-H**	0.020	**1-H**	1.029	**3-H**	-0.174	**1-H**	-0.229
1-H	-0.413	**5-H**	-0.856	**1-H**	0.896	**4-H**	0.472	**2-H**	0.020	**7-H**	1.385	**4-H**	0.040	**3-H**	-0.176
2-H	-0.360	**3-H**	-0.824	**4-H**	1.333	**1-H**	0.605	**5-H**	0.042	**8-H**	1.767	**1-H**	0.262	**2-H**	0.177
3-H	-0.344	**1-H**	-0.485	**7-H**	1.721	**3-H**	0.684	**6-H**	0.146	**6-H**	1.865	**2-H**	0.402		
4-H	0.081	**2-H**	-0.403	**6-H**	1.724	**2-H**	1.081	**4-H**	0.184	**4-H**	2.025				
				5-H	1.814	**5-H**	1.403	**3-H**	0.819	**5-H**	2.100				
				3-H	2.597	**7-H**	2.585			**2-H**	2.184				
										3-H	2.451				
Marvinbeans															
1c		**2ec**		**3cg**		**4ecg**		**5egc**		**6egcg**		**7ga**		**8caffa**	
1-H	6.580	**1-H**	5.974	**1-H**	6.418	**1-H**	6.451	**1-H**	6.202	**1-H**	6.362	**2-H**	1.674	**3-H**	2.345
5-H	7.523	**4-H**	6.518	**4-H**	6.707	**6-H**	6.574	**4-H**	7.164	**6-H**	6.948	**3-H**	5.979	**2-H**	6.854
2-H	8.896	**2-H**	7.531	**7-H**	6.826	**4-H**	6.994	**6-H**	7.164	**7-H**	7.094	**4-H**	7.764	**1-H**	7.986
4-H	9.278	**5-H**	8.790	**6-H**	6.991	**3-H**	7.011	**5-H**	8.070	**8-H**	7.161	**1-H**	7.866		
3-H	12.364	**3-H**	12.404	**3-H**	7.139	**7-H**	7.277	**2-H**	8.134	**4-H**	7.173				
				5-H	8.842	**5-H**	8.773	**3-H**	12.312	**3-H**	7.183				
				2-H	8.897	**2-H**	9.035			**5-H**	8.124				
										2-H	9.013				
Sparc															
1c		**2ec**		**3cg**		**4ecg**		**5egc**		**6egcg**		**7ga**		**8caffa**	
4-H	8.790	**4-H**	8.790	**6-H**	6.690	**6-H**	6.690	**4-H**	8.610	**6-H**	6.690	**2-H**	3.980	**3-H**	4.400

Table 5. (Continued) pK_a order belonging to the -OH group protons in the structure of molecules calculated from the different methods

2-H	9.090	**2-H**	9.090	**3-H**	7.980	**3-H**	7.980	**6-H**	8.610	**3-H**	7.980	**4-H**	6.910	**1-H**	7.890
5-H	9.360	**5-H**	9.360	**7-H**	7.980	**7-H**	7.980	**5-H**	8.840	**7-H**	7.980	**1-H**	8.590	**2-H**	9.070
1-H	9.680	**1-H**	9.680	**2-H**	8.550	**2-H**	8.550	**2-H**	8.990	**4-H**	8.380	**3-H**	8.590		
3-H	14.900	**3-H**	15.040	**5-H**	8.560	**5-H**	8.560	**1-H**	9.680	**8-H**	8.380				
				4-H	9.280	**4-H**	9.280	**3-H**	15.220	**2-H**	8.440				
				1-H	9.500	**1-H**	9.500			**5-H**	8.760				
										1-H	9.500				

3. COMPUTATIONAL METHOD

The CS Chemoffice and the MOPAC2009 programs were used for the calculation of the physicochemical properties of the tea components. All the molecules were drawn by the CS ChemDraw and then minimized by the MM2 method in the CS Chem 3D program (MOPAC 2009). Their theoretical calculations in aqueous and blood phases were carried out using the new semi-empirical PM6 quantum chemical methods in the MOPAC2009 program. The pK_a values of the molecules were computed by MarvinSketch v5.3.1 (ChemAxon) and SPARC v4,5 (SPARC 2009) programs in aqueous and blood phases and the results were compared with those of experimental results.

The most stable conformations of all of the studied molecules were computed by all the present computational methods.

4. CONCLUSION

It is thought that not only the number of –OH group but also the geometry of the tea components has an important role in the reactivities of these compounds in human metabolism. Dipole moment and nucleophilicity did not give any important information from the viewpoint of the reactivity for these molecules. The best correlations could be observed between PM6 and experimental pK_a values for **6egcg and 8caffa**; PM6-ΔG_f for **4ecg**; Marvin-experimental for **1c and 2ec;** ΔG_f-Sparc for **3cg**, ΔG_f-ΔG for **5egc** and Marvinbeans-Sparc for **7ga** in the aqueous phase; and Marvinbeans-experimental for **1c** and **2ec**; ΔG_f-Marvinbeans for **3cg**; ΔG-experimental for **4ecg**; ΔG_f-Sparc for **5egc**; ΔG-Sparc for **6egcg**; and Marvinbeans-Sparc for **7ga** and ΔG_f-experimental for **8caffa** in the blood phase.

Positive meaningful correlations ($R^2 > 0.9999$) were observed between thermokinetic pK_a values in both aqueous and blood phases for **1c, 2ec, 3cg**, **4ecg**; **5egc, 6egcg** and **7ga**. It can be resulted that the mentioned molecules have showed similar behaviors like permeability in the blood phase with those molecules in the aqueous phase due to their good correlations.

A good correlation could not be observed among the pK_a values for the similar acidic proton belonging to the –OH in the molecules for all calculation methods [PM6, ΔG_f (Thermokinetic), ΔG (Thermodynamic), Marvinbeans and Sparc].

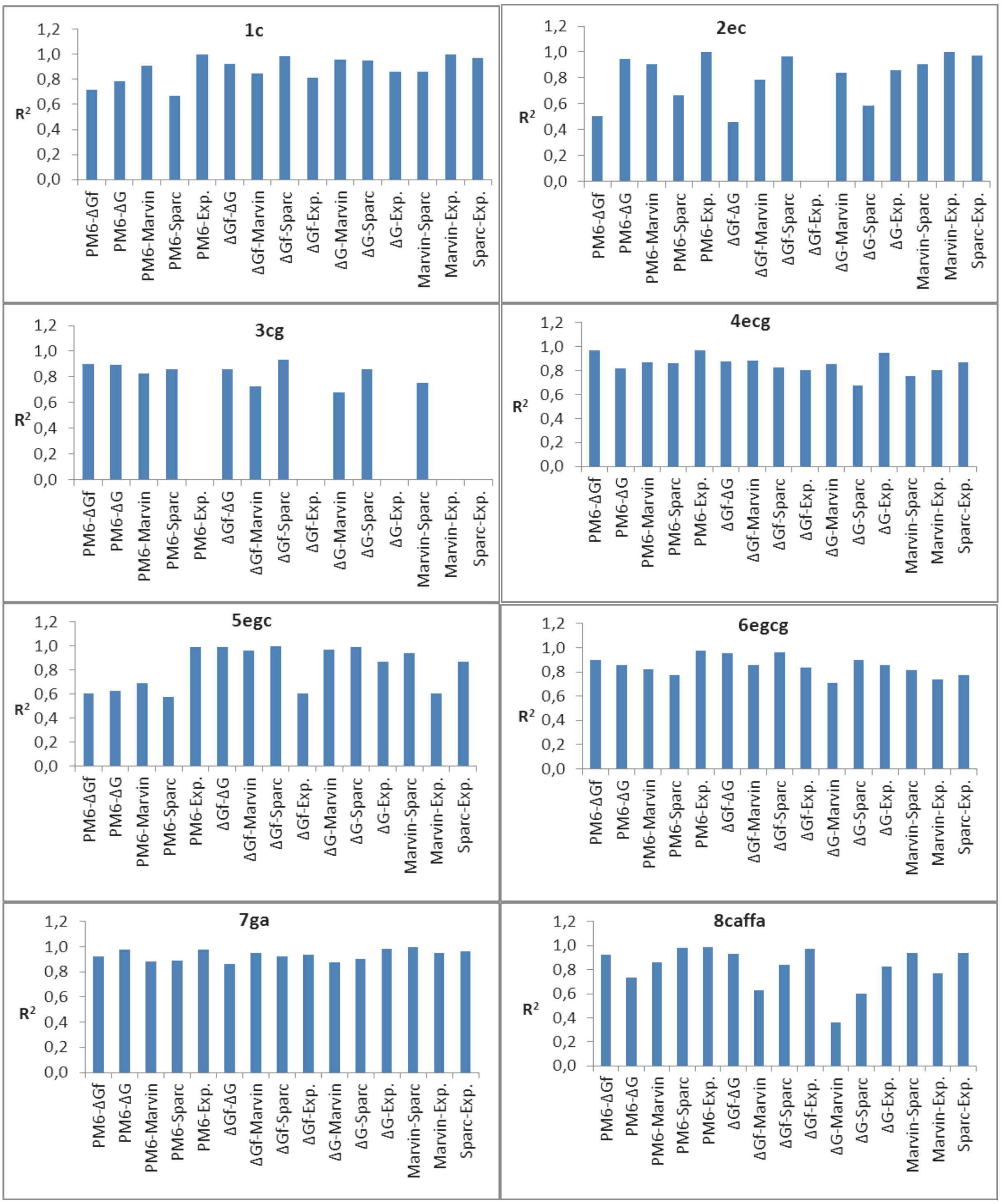


Figure 2. The correlations for pK_a values in the aqueous phase by different calculation methods [experimental: Tama et al. 2001; Adams et al. 2002; Inoue et al. 2002; Herrero-Martínez et al. 2005), PM6, ΔG_f (Thermokinetic), ΔG (Thermodynamic), Marvinbeans and Sparc], R^2: Correlation coefficient.

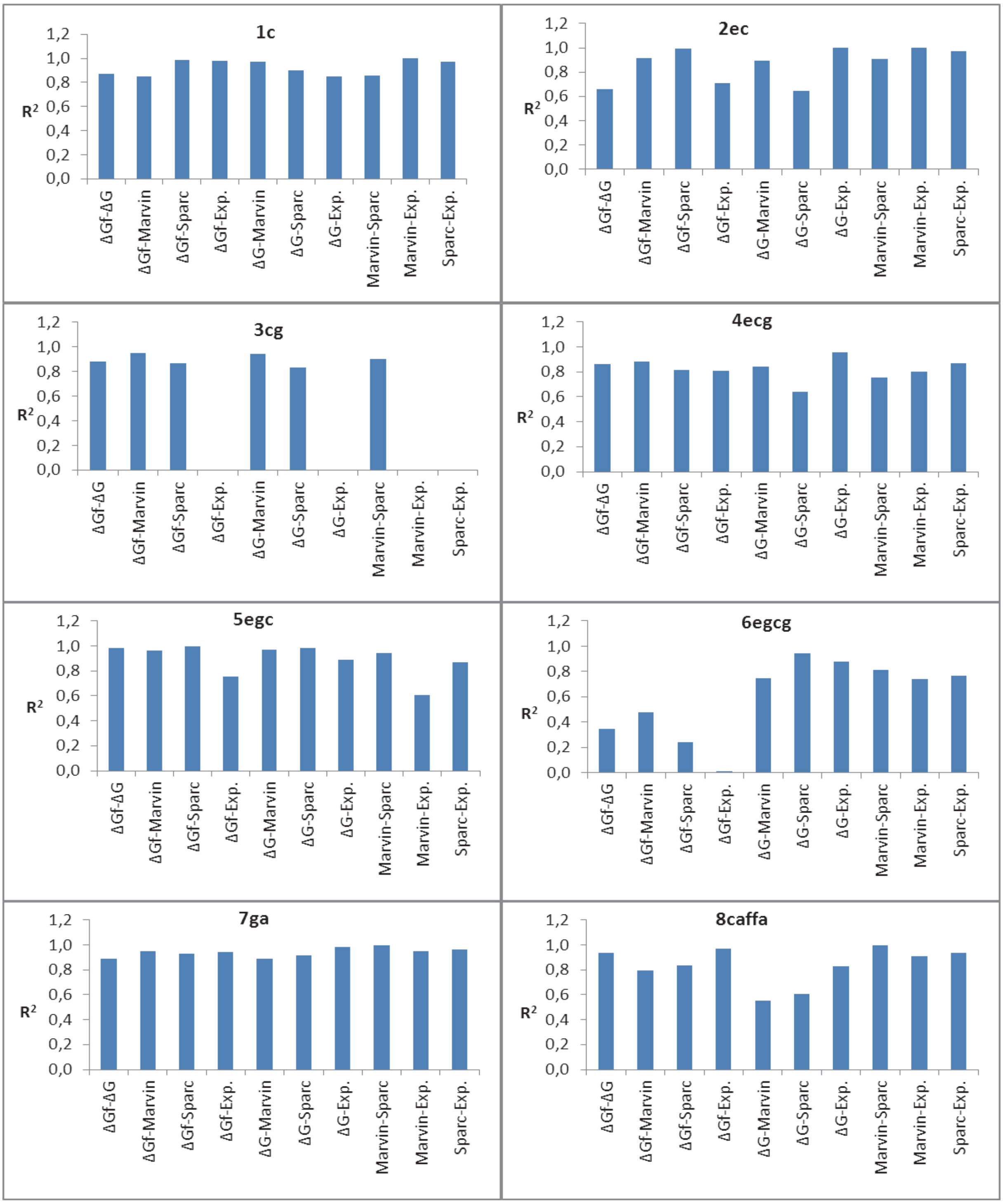


Figure 3. The correlations for pK_a values in the blood phase by different calculation methods [experimental: Tama et al. 2001; Adams et al. 2002; Inoue et al. 2002; Herrero-Martínez et al. 2005), PM6, ΔG_f (Thermokinetic), ΔG (Thermodynamic), Marvinbeans and Sparc], R^2: Correlation coefficient.

ACKNOWLEDGEMENTS

The authors are grateful for being permitted to access MOPAC2009, Marvinbeans and Sparc computer programs.

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